

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property
Organization
International Bureau



(43) International Publication Date
12 August 2004 (12.08.2004)

PCT

(10) International Publication Number
WO 2004/067775 A3

(51) International Patent Classification⁷: C12Q 1/68, 1/68

(21) International Application Number:
PCT/EP2004/000856

(22) International Filing Date: 30 January 2004 (30.01.2004)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
103 04 219.9 30 January 2003 (30.01.2003) DE

(71) Applicant (for all designated States except US): EPIGENOMICS AG [DE/DE]; Kastanienallee 24, 10435 Berlin (DE).

(72) Inventor; and

(75) Inventor/Applicant (for US only): BERLIN, Kurt [DE/DE]; Marienkäferweg 4, 14532 Stahnsdorf (DE).

(74) Agent: SCHUBERT, Klemens; Neue Promenade 5, 10178 Berlin (DE).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN,

CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

- with international search report
- before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments

(88) Date of publication of the international search report:
9 December 2004

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: METHOD FOR THE DETECTION OF CYTOSINE METHYLATION PATTERNS WITH HIGH SENSITIVITY

(57) Abstract: The present invention concerns a method for the detection of cytosine methylation in DNA samples, in which the following steps are conducted: a genomic DNA sample which comprises target DNA and background DNA is chemically treated such that all unmethylated cytosine bases are converted to uracil, while the 5-methylcytosine bases remain unchanged; the chemically treated DNA sample is amplified with the use of at least 2 primer oligonucleotides as well as a polymerase and a nucleotide mixture, the composition of which leads to a preference for the target DNA over the background DNA as the template; and the methylation state in the target DNA is concluded from the presence of an amplificate or its quantity.

WO 2004/067775 A3

INTERNATIONAL SEARCH REPORT

International Application No

EP2004/000856

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12Q1/68 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS, WPI Data, PAJ, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 6 265 171 B1 (HERMAN JAMES G ET AL) 24 July 2001 (2001-07-24)	1,11,12, 14,21, 22,30, 37-40
Y	the whole document	2-10,13, 15-20, 23-29, 31-36
X	WO 01/44504 A (FOX JAYNE CATHERINE ; HAQUE KEMAL (GB); LITTLE STEPHEN (GB); ASTRAZENE) 21 June 2001 (2001-06-21)	1,11,12, 14,21, 22,30, 37-40
Y	the whole document	2-10,13, 15-20, 23-29, 31-36

-/--

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents:

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

Z document member of the same patent family

Date of the actual completion of the international search

29 September 2004

Date of mailing of the international search report

21/10/2004

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax (+31-70) 340-3016

Authorized officer

Botz, J

INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP2004/000856

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 00/70090 A (UNIV SOUTHERN CALIFORNIA) 23 November 2000 (2000-11-23) cited in the application	1, 11, 12, 14, 21, 22, 30, 37-40
Y	the whole document	2-10, 13, 15-20, 28, 29, 31-36
X	OLEK ALEXANDER ET AL: "A modified and improved method for bisulphite based cytosine methylation analysis" NUCLEIC ACIDS RESEARCH, vol. 24, no. 24, 1996, pages 5064-5066, XP002106408 ISSN: 0305-1048 cited in the application the whole document	1, 11-14, 21, 22, 30, 37-40
Y	WORM JESPER ET AL: "In-tube DNA methylation profiling by fluorescence melting curve analysis" CLINICAL CHEMISTRY, vol. 47, no. 7, July 2001 (2001-07), pages 1183-1189, XP002298308 ISSN: 0009-9147 the whole document	10
Y	WO 99/60007 A (HAMILTON ALAN LEWIS ; SHCHEPINOV MIKHAIL SERGEEVICH (GB); SOUTHERN EDW) 25 November 1999 (1999-11-25) the whole document	31-36
Y	WO 97/37041 A (SEQUENOM INC) 9 October 1997 (1997-10-09) the whole document	31-36
Y	OLEJNIK J ET AL: "Photocleavable peptide-DNA conjugates: synthesis and applications to DNA analysis using MALDI-MS" NUCLEIC ACIDS RESEARCH, OXFORD UNIVERSITY PRESS, SURREY, GB, vol. 27, no. 23, 1 December 1999 (1999-12-01), pages 4626-4631, XP002220753 ISSN: 0305-1048 the whole document	31-36
Y	WO 01/76451 A (PIEPENBROCK CHRISTIAN ; BERLIN KURT (DE); EPIGENOMICS AG (DE); OLEK AL) 18 October 2001 (2001-10-18) the whole document	15-18, 31-36
	-/--	

INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP2004/000856

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	<p>YU ET AL: "SPECIFIC INHIBITION OF PCR BY NON-EXTENDABLE OLIGONUCLEOTIDES USING A 5' TO 3' EXONUCLEASE-DEFICIENT DNA POLYMERASE" BIOTECHNIQUES, EATON PUBLISHING, NATICK, US, vol. 23, no. 4, October 1997 (1997-10), pages 714,716-720, XP001041499 ISSN: 0736-6205 the whole document</p>	19,20
Y	<p>US 5 866 336 A (NAZARENKO IRINA A ET AL) 2 February 1999 (1999-02-02) the whole document</p>	23-27
Y	<p>WO 00/46398 A (KAY PETER H ; UNIV WESTERN AUSTRALIA (AU)) 10 August 2000 (2000-08-10) the whole document</p>	23-27
A	<p>AUER T ET AL: "SELECTIVE AMPLIFICATION OF RNA UTILIZING THE NUCLEOTIDE ANALOG DITPAND THERMUS THERMOPHILUS DNA POLYMERASE" NUCLEIC ACIDS RESEARCH, OXFORD UNIVERSITY PRESS, SURREY, GB, vol. 24, no. 24, 1996, pages 5021-5025, XP002916438 ISSN: 0305-1048 the whole document</p>	2-9

INTERNATIONAL SEARCH REPORT

International application No.

PCT/EP2004/000856

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.b of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, the international search was carried out on the basis of:
 - a. type of material
 - ☒ a sequence listing
 - ☐ table(s) related to the sequence listing
 - b. format of material
 - ☒ In written format
 - ☒ In computer readable form
 - c. time of filing/furnishing
 - ☐ contained in the international application as filed
 - ☐ filed together with the international application in computer readable form
 - ☒ furnished subsequently to this Authority for the purpose of search
2. ☒ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional comments:

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/EP2004/000856

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
US 6265171	B1	24-07-2001	US 6017704 A	25-01-2000
			US 5786146 A	28-07-1998
			CA 2257104 A1	11-12-1997
			EP 0954608 A1	10-11-1999
			IL 127342 A	25-07-2002
			JP 2000511776 T	12-09-2000
			WO 9746705 A1	11-12-1997
			US 6200756 B1	13-03-2001
WO 0144504	A	21-06-2001	AU 2194801 A	25-06-2001
			WO 0144504 A2	21-06-2001
WO 0070090	A	23-11-2000	US 6331393 B1	18-12-2001
			AU 775798 B2	19-08-2004
			AU 4712200 A	05-12-2000
			CA 2372665 A1	23-11-2000
			EP 1185695 A1	13-03-2002
			JP 2002543852 T	24-12-2002
			WO 0070090 A1	23-11-2000
			US 2002086324 A1	04-07-2002
WO 9960007	A	25-11-1999	AT 256142 T	15-12-2003
			AU 3943799 A	06-12-1999
			CA 2332862 A1	25-11-1999
			DE 69913509 D1	22-01-2004
			DE 69913509 T2	23-09-2004
			DK 1068216 T3	13-04-2004
			EP 1428810 A2	16-06-2004
			EP 1068216 A2	17-01-2001
			WO 9960007 A2	25-11-1999
			JP 2002515588 T	28-05-2002
			US 6780981 B1	24-08-2004
WO 9737041	A	09-10-1997	US 6194144 B1	27-02-2001
			AU 2217597 A	22-10-1997
			WO 9737041 A2	09-10-1997
			US 6238871 B1	29-05-2001
WO 0176451	A	18-10-2001	DE 10019058 A1	20-12-2001
			DE 10032529 A1	07-02-2002
			AU 4835201 A	24-09-2001
			AU 5038101 A	24-09-2001
			AU 5478801 A	23-10-2001
			AU 5479401 A	23-10-2001
			AU 7384001 A	23-10-2001
			AU 7566301 A	23-10-2001
			AU 7633001 A	23-10-2001
			AU 7633101 A	23-10-2001
			AU 7748701 A	23-10-2001
			AU 7842001 A	07-11-2001
			AU 8960001 A	11-12-2001
			WO 0177373 A2	18-10-2001
			DE 20121960 U1	15-01-2004
			DE 20121964 U1	15-01-2004
			DE 20121965 U1	24-12-2003
			DE 20121968 U1	18-12-2003
			DE 20121969 U1	24-12-2003
			DE 20121970 U1	24-12-2003

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/EP2004/000856

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 0176451	A		DE 20121972 U1	24-12-2003
			DE 20121973 U1	24-12-2003
			DE 20121974 U1	24-12-2003
			DE 20121977 U1	24-12-2003
			WO 0168911 A2	20-09-2001
			WO 0168912 A2	20-09-2001
			WO 0177375 A2	18-10-2001
			WO 0177164 A2	18-10-2001
			WO 0177376 A2	18-10-2001
			WO 0177377 A2	18-10-2001
			WO 0181622 A2	01-11-2001
			WO 0192565 A2	06-12-2001
			WO 0177378 A2	18-10-2001
			WO 0176451 A2	18-10-2001
			EP 1283905 A2	19-02-2003
			EP 1268855 A2	02-01-2003
			EP 1268857 A2	02-01-2003
			EP 1272670 A2	08-01-2003
			EP 1278892 A1	29-01-2003
			EP 1278893 A2	29-01-2003
			EP 1274865 A2	15-01-2003
			EP 1274866 A2	15-01-2003
			EP 1360319 A2	12-11-2003
			EP 1370685 A2	17-12-2003
			EP 1268861 A2	02-01-2003
			JP 2004507213 T	11-03-2004
			JP 2004507214 T	11-03-2004
			JP 2004508807 T	25-03-2004
			JP 2003534780 T	25-11-2003
			JP 2003531589 T	28-10-2003
US 5866336	A	02-02-1999	AU 3728597 A	09-02-1998
			CA 2260973 A1	22-01-1998
			EP 0912597 A1	06-05-1999
			JP 2001513623 T	04-09-2001
			JP 2003111599 A	15-04-2003
			WO 9802449 A1	22-01-1998
			US 6117635 A	12-09-2000
			US 6090552 A	18-07-2000
WO 0046398	A	10-08-2000	WO 0046398 A1	10-08-2000
			AU 761763 B2	12-06-2003
			AU 2648000 A	25-08-2000
			CA 2361654 A1	10-08-2000
			EP 1151138 A1	07-11-2001
			JP 2002535998 T	29-10-2002
			US 2003017465 A1	23-01-2003